British Biotechnology Journal
14(3): 1-10, 2016, Article no.BBJ.26488
ISSN: 2231–2927, NLM ID: 101616695

SCIENCEDOMAIN *international*
www.sciencedomain.org

Determination of Phenolic Contents, Antioxidant and Antibacterial Activities of Strawberry Tree (*Arbutus unedo* L.) Leaf Extracts

Abdelhakim Bouyahya[1,2*], Naima El Moussaoui[2], Jamal Abrini[2], Youssef Bakri[1] and Nadia Dakka[1]

[1]Department of Biology, Biochemistry-Immunology Laboratory, Faculty of Sciences, Mohammed V University, Rabat, Morocco.
[2]Department of Biology, Biology and Health Laboratory, Team of Biotechnology and Applied Microbiology, Faculty of Science, Abdelmalek Essaadi University, Tetouan, Morocco.

Authors' contributions

This work was carried out in collaboration between all authors. Study design was suggested by authors ND and JA. Author AB has performed the overall practical study, interpreted and wrote the first draft of the manuscript. Authors ND, YB and JA have designed the protocol of antibacterial and antioxidant activities. Author NEM provided technical advices. Authors AB, NEM and ND analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2016/26488
Editor(s):
(1) Nikolaos Labrou, Department of Agr. Biotechnology, Agricultural University of Athens, Greece.
Reviewers:
(1) Abdullah Ijaz Hussain, Institute of Chemistry, Government College University Faisalabad, Faisalabad, Pakistan.
(2) Ana Margarida Violante Pombo, Polytechnic Institute of Leiria, Portugal.
(3) Patcharin Raviyan, Chiang Mai University, Thailand.
(4) O. E. Yakubu, Federal University Wukari, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/15247

Original Research Article

Received 20th April 2016
Accepted 21st June 2016
Published 1st July 2016

ABSTRACT

Aims: This study was designed to evaluate the antibacterial and antioxidant activities of methanolic, ethanolic, ethyl acetate and *n*-hexanic extract from the leaves of Strawberry tree (*Arbutus unedo* L.) growing in Morocco.
Study Design: Determination of total phenol and flavonoids content, *in vitro* antioxidant activity and antibacterial study of extracts.
Place and Duration of Study: Department of Biology (Faculty of Sciences), between July 2015 and November 2015.

**Corresponding author: E-mail: boyahyaa-90@hotmail.fr;*

Methodology: The extracts were prepared using solid-liquid extraction. The total phenolic content was assessed by the Folin-Ciocalteau assay, total flavonoid content was assessed by aluminium chloride (AlCl3) colorimetric assay. The antibacterial activity of extracts was tested against five reference strains, *Escherichia coli* K12 MBLA, *Staphylococus aureus* CECT 976, *Listeria monocytogenes serovar* 4b CECT 4032 and *Pseudomonas aeruginosa* IH using the agar well diffusion method and the micro-dilution assays. The antioxidant activity was assessed by measuring the ability of the extracts to scavenge the 2,2-diphenyl-1- picrylhydrazyl (DPPH).
Results: The total phenol content of strawberry tree five extracts ranged between 94.51±0.08 and 141.72±0.56 mg GAE/g extract, and the flavonoid content ranged between 17.48±1.02 and 31.61±0.59 mg QE/g extract. All of the extracts presented antioxidant capacity assessed by DPPH scavenging method, but at different levels depending on the concentration and the extraction solvent are found. In addition, all extracts exhibited an antibacterial activity at different levels against strains reported as the causal agents of food borne diseases.
Conclusion: Results suggest the potential use of tested *A. unedo* extracts as bio-preservatives in the functional food industry.

Keywords: Arbutus unedo; antibacterial activity; antioxidant activity; phytochemical screening.

1. INTRODUCTION

Oxidation is the transfer of electrons from one atom to another and represents an essential part of both aerobic life and our metabolism, since oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of ATP. However, problems may arise when the electron flow becomes uncoupled (transfer of unpaired single electrons), generating free radicals [1]. Antioxidants are important in living organisms as well as in food because they may delay or stop formation of free radical by giving hydrogen atoms or scavenging them. Oxidative stress is involved in the pathology of cancer, atherosclerosis, malaria and rheumatoid arthritis. An antioxidant can be defined in the broadest sense of the word, as any molecule capable of preventing or delaying oxidation (loss of one or more electrons) from other molecules, usually biological substrates such as lipids, proteins or nucleic acids [2,3].

The bacterial organisms including gram positive and gram negative like different species of *Escherichia, Staphylococcus, Listeria and Pseudomonas* are the main source to cause severe infections in humans [4]. Resistance to antimicrobials is a significant and growing problem, limiting treatment options, especially for serious infections. These are the major cause of worldwide outbreaks of both hospitals and the community infections [5]. The spread of multidrug-resistant (MDR) strains of bacteria necessitates the discovery of new classes of antibacterials and compounds that inhibit these resistance mechanisms [6].

The use of traditional medicine is widespread in Morocco and plants are indeed the first source for preparing remedies in this form of alternative medicine. The North-west of Morocco (Ouezzane province) is rich in medicinal and a few studies was carried-out of them reveled antibacterial activity [7]. Among the various compounds found in plants, antioxidants are of particular importance because they might serve as leads for the development of novel drugs. On other hand the use of some plants in nutriment system can prevent and/or protect against diseases [8]. Several plants used as anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective properties have recently been shown to have and antioxidant and/or antiradical scavenging mechanism as part of their activity [9,10]. The search for natural sources of medicinal products that also have antioxidant and antibacterial activities is on the rise [7,11,12].

Arbutus unedo L. that belongs to *Ericaceae family's* is an evergreen shrub or small tree, and it is widely distributed in the Mediterranean region and in the North Africa. It is widely used in traditional medicine in Morocco as astringent, urinary tract antiseptic, anti-diarrheal and depurative properties [13,14].

Several studies have shown the biological properties of *A. unedo*. Others studies showed that an aqueous extract of *A. unedo* exhibited antihypertensive [15,16], while Rosato et al. [17] showed vaso-relaxant properties. Furthermore, an *in vitro* study indicated that diethyl-ether and ethyl acetate extracts of *A. unedo* leaves have an anti-aggregating effect on human platelets

[17,18]. This effect is likely mediated by its antioxidant activity [18-20].

We report here the antimicrobial and antioxidant properties and the phenols content of five extracts of *A. unedo* (leaves). The antibacterial activity of extracts was evaluated on five reference strains, *Escherichia coli* K12 MBLA, *Staphylococus aureus* CECT 976, *Listeria monocytogenes serovar* 4b CECT 4032 and *Pseudomonas aeruginosa* IH. We determined the zone of growth inhibition, minimum inhibitory and bactericidal concentrations (MIC and MBC). The antioxidant activity was tested by using scavenging DPPH method and the IC_{50} was determined graphically.

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaves of *A. unedo* used in this research were collected in July 2015 North-West of Morocco from Ouezzane Province (34° 47' 50" N and 5° 34' 56" W). Plant was authenticated by Pr. ENNABILI Abdesalam (National Institute of Medicinal and Aromatic Plants, Taounate, Morocco). Samples were further transported to the laboratory. Samples were air dried under the shade and then milled into powder (using an electric grinder) for extraction.

2.2 Extraction

The investigated dried powdered plant materials were extracted by maceration. The powder (25 g) of leaves was placed in an Erlenmeyer flask in 100 ml ethanol (EtOH), methanol (MeOH), and *n*-hexane for 72 h. The plant extracts were filtered by Whatman No. 1 filter paper and the combined filtrate was then dried under vacuum using a rotary evaporator (Heidolph Collegiate, LV28798826, New Jersey, USA) at a temperature not exceeding 45℃. The methanol concentrated extract was dissolved in distilled water and extracted with ethyl acetate to obtain ethyl acetate fraction (EtOAc). All extracts were stored in a dark bottle for investigation at 2 - 8℃.

2.3 Determination of Total Phenolic Content (TPC)

The concentration of the phenolic in the plants extracts was determined using the Folin Ciocalteu assay [21], with some modifications. In brief, the extract was diluted to the concentration of 1 mg mL^{-1}, and aliquots of 100 µl or a standard solution of gallic acid (20, 40, 60, 80 and 100 mg L^{-1}) were mixed with 500 µl of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 400 µl of Na_2CO_3 (7%). After 40 min of incubation at room temperature (23±2), the absorbance was measured at 760 nm using a Spectro-photometer (J.P. Selecta, sa 4120007, Barcelona, Spain) against a blank sample. The total phenolic content was calculated using a calibration curve for gallic acid (R^2= 0.998). The results were expressed as the gallic acid equivalent per gram of dry weight of extract (mg of GAE/g of extract). Alls samples were analyzed in triplicate.

2.4 Determination of Total Flavonoids Contents (TFC)

The total flavonoid content of the extracts was determined using the aluminium chloride ($AlCl_3$) colorimetric method described by Brighente et al. [22] with minor modifications. Briefly, 1 mL of the extract (1 mg mL^{-1} in methanol) or a standard solution of quercetin (20, 40, 60, 80 and 100 mg L^{-1}) was mixed with 1 ml of 2% AlCl3 in methanol. After 40 min of staying at room temperature (23±2℃), the absorbance against blank was measured at 430 nm using a Spectrophotometer (J.P. Selecta, sa 4120007, Barcelona, Spain). The total flavonoid content was calculated using a calibration curve for quercetin (R^2= 0.985). The results were expressed as the quercetin equivalent per gram of dry weight of extract (mg of QE/g of extract). All samples were analyzed in triplicate.

2.5 Antioxidant Activity

2.5.1 Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay

The ability of the plant extracts to scavenge DPPH free radicals was assessed using the standard method with some modifications [23]. In brief, Aliquots (0.2 mL) of various concentrations (30,125–1000 µg mL^{-1}) of the plant extracts samples were added to 1,8 mL of a 0.004% methanolic solution of DPPH. After an incubation period of 30 min in darkness at room temperature (23±2℃), the absorbance was recorded against a blank at 517 nm with a spectrophotometer. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the control. Samples were analyzed in triplicate.

$$\%\ Inihiition = \frac{Abs\ (blank) - Abs(sample)}{Abs\ (blank)} \times 100$$

Where *A*bs (blank) is the absorbance of the control and *Abs (sample)* is the absorbance of the sample.

Trolox and ascorbic acid was used as positive control and the concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against the extract concentration. Relationships between antioxidant capacity (IC_{50}) and total phenol content or flavonoid content of extract were evaluated by correlation coefficients. This correlation was determined by modeling the antioxidant capacity versus total phenol content or flavonoids content.

2.6 Antibacterial Activity

2.6.1 Bacteria strains

In order to evaluate the antibacterial activity of the various extract of *A. unedo*, the flowing bacteria were used: *Escherichia coli* K12 MBLA (Laboratory of Food Microbiology, UCL, Belgium: MBLA), *Staphylococus aureus* CECT 976, *Listeria monocytogenes serovar* 4b CECT 4032 (Spanish Type Culture Collection: CECT), and *Pseudomonas aeruginosa* IH (Institute of hygiene, Rabat, Morocco: IH). Strains are maintained on an inclined agar medium at 4℃. Before use, the bacteria were revived by two subcultures in an appropriate culture medium: Lysogeny broth (LB) (Biokar Diagnostics, Beauvais, France) at 37℃ for 18 to 24 h. For the test, final inoculums concentrations of 10^6 CFU mL^{-1} bacteria were used according to the National Committee for Clinical Laboratory Standards, USA (NCCLS 1999).

2.6.2 Antibacterial susceptibility test: Agar-well diffusion assay

A basal layer was prepared by Muller-Hinton agar. After the agar plates were solidified, sterile 8 mm diameter cylinders were deposited. Six ml of LB medium in superfusion containing 0.8% agar were inoculated by a fresh culture of indicator bacterial strain (a final concentration was 10^6 CFU mL^{-1}). After solidification, the wells were filled with 50 µl of diluted extracts at 25 mg mL^{-1}. After incubation at appropriate temperature for 24 h, all plates were examined for any zone of growth inhibition, and the diameter of these zones was measured in millimeters. All the tests were performed in triplicate.

2.6.3 Determination of minimal inhibitory concentration (MIC)

MICs were determined using the broth micro-dilution assay, as previously described by Ismaili et al. [24], with a slight modification: agar at 0.15% (w/v) was used as stabilizer of the extract–water mixture and resazurin as bacterial growth indicator. 50 µl of Bacteriological Agar (0.15% w/v) was distributed from the 2nd to the 8th well of a 96-well polypropylene microtitre plate. A dilution of the each extract was prepared in DMSO (10%), to reach a final concentration of 32 mg mL^{-1}; 100 µl of these suspensions was added to the first test well of each microtitre line, and then 50 µl of scalar dilution was transferred from the 2nd to the 7th well. The 8th well without extract added was considered as growth control. Then, we added 50 µl of a bacterial suspension to each well at a final concentration of approximately 10^6 CFU mL^{-1}. The final concentration of the extract was between 16 and 0.25 mg L^{-1}. After incubation at 37℃ for 18 h, 10 µl of resazurin was added to each well to assess bacterial growth. After further incubation at 37℃ for 2 h, the MIC was determined as the lowest essential oil concentration that prevented a change in resazurin color. Bacterial growth was detected by reduction in blue dye resazurin to pink resorufin. A control was carried out to ensure that, at the concentrations tested, the extract did not cause a color change in the resazurin. Experiments were performed in triplicate.

2.6.4 Determination of minimal bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) corresponded to the lowest concentration of the essential oil yielding negative subcultures after incubation at appropriate temperature for 24 h. It is determined in broth dilution tests by sub-culturing 10 µl from negative wells on plate count agar (PCA) medium. All the tests were performed in triplicate.

2.7 Data Analysis

The statistical analysis was performed by a one-way ANOVA analysis of variance followed by Duncan's test, and results were considered to be statistically significant with a 95% confidence level ($p < 0.05$). The measurements of total phenolic compounds, total flavonoids, DPPH radical scavenging activity, and antibacterial activity were carried out for three replicates. The results are expressed as mean ± SD.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content

Total phenol content was estimated by the Folin-Ciocalteu colorimetric method in comparison with standard gallic acid and the results were expressed in terms of mg GAE/g extract. The organic extracts of *A. unedo* had an important charge of phenols and their values varied widely for both used organic solvent. Among the extracts investigated, total phenolic content ranged from 94.51±0.08 to 141.72±0.56 gallic acid equivalents (GAE mg/g extract) (Table 1). The methanol extracts showed highest phenol content (141.72±0,56 GAE mg/g extract). While n-hexane extracts have the lowest phenol content by 94.51±0,08 GAE mg/g extract).

Phenolic compounds are secondary metabolites that can act as antioxidant agents that have been widely investigated in many medicinal plants, fruits, and vegetables [25]. This antioxidant activity due to their redox properties [26], which play an important role in adsorbing and neutralizing free radicals [27], to donate hydrogen, quench singlet oxygen, act as metal chelators [28] and decomposing peroxides [29].

Ours results concerning phenols contents are higher than other study that have reported the phenols compounds leaves extracts of *A. unedo* collected from Montenegro [30]. Others searches carried out in Croatia have shown that leaves extract are rich in phenols compounds and reported that the concentrations of these compounds changed over the year [31]. The highest concentration of total phenols content could be explained by the Mediterranean climate of North-West of Morocco that can induce modulations of the secondary metabolism pathways of plants.

3.2 Total Flavonoids

The total flavonoid content was estimated by a colorimetric method using quercetin as standard flavonoid. As in the case of total phenolic content, the concentration of flavonoids in the extracts was dependent on solvent extract. Their values ranging from 17.48±1.02 to 31.61±0.59 quercetin equivalents of dry weight of extract (QE mg/g extract) (Table 1). As in the case of total phenolic content, the methanolic extracts showed higher ($p < 0.05$) flavonoids content (31.61±0.59 mg QE/g extract) than the other extracts, which showed similar levels ($p > 0.05$). While, on the contrary of the case of total phenol content, the ethyl acetate extracts had the lowest yielded of flavonoid content (17.48±1.02 mg QE/g extract), but no significantly difference ($p > 0,05$) has detected between flavonoid content of ethanol, *n*-hexane and ethyl acetate extract. These effects are related with an antioxidant potential of flavonoids. Our result are in consonance with other study reported by Mendes et al. [32] who have revealed that leaf extracts of *A. unedo* collected from the Natural Park of Montesinho (Bragança, Northeast of Portugal) were shown to have highest concentration of flavonoids.

3.3 Antioxidant Activity

The reduction capability of DPPH radicals was determined by the antioxidant-induced decreases in its absorbance at 517 nm, which is visually noticeable as a discoloration from purple to yellow. This average absorbance was used to calculate the DPPH scavenging capacity. Therefore, DPPH was used as a substrate to evaluate the antioxidant activity of methanol, ethanol, n-hexane and ethyl-acetate extracts of *A. unedo* from leaves at various concentrations (0-500 µg mL^{-1}). All the plant extracts were found to be capable of scavenging DPPH radicals. Fig. 1 shows the results obtained, a significant decrease in the concentration of DPPH radical due to the scavenging ability of *A. unedo* extracts and standards was detected. Indeed, at 60.25 µg mL^{-1}, the ascorbic acid and Trolox showed a high radical scavenging activity (100 and 98.29%, respectively), while the activity of the methanol, ethanol, n-hexane and ethyl-acetate extracts were respectively 39.42, 18.19, 47.53 and 19.52 %. The various extracts activity was significantly lower than ascorbic acid and Trolox ($p < 0.05$). In all case, we have found that the activity to scavenge DPPH radical increases significantly with increasing extract concentration ($p < 0.05$).

Our results revealed that n-hexane and methanol extract showed the highest inhibition in all concentration than ethyl-acetate and ethanol extract ($p < 0.01$). In the other hand, none significantly difference between n-hexane and methanol extract activity was detected ($p > 0.01$). From this assay, it is clear that the n-hexane and methanol extracts of leaves from *A. unedo* showed the highest radical scavenging activity.

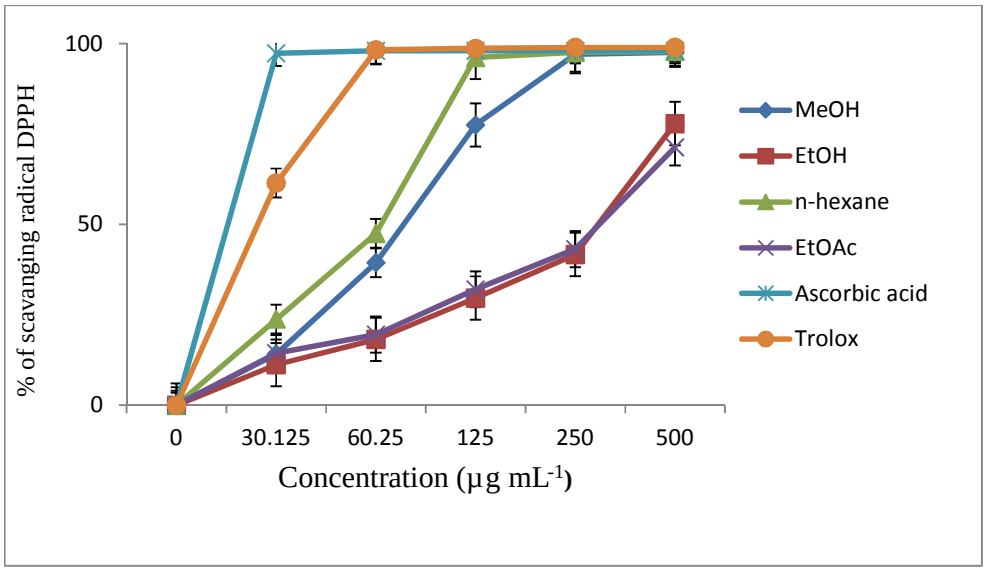

Fig. 1. DPPH radical scavenging activities (%) of standard antioxidants (Trolox and ascorbic acid) and *Arbutus unedo extracts* (MeOH: Methanolic extract, EtOH: Ethanolic extract, EtOAc: Ethyl acetate extract and *n*-hexanic extract)

Values are means ± standard deviation of three determinations

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) of *A. unedo* extracts

	Extracts			
	MeOH	**EtOH**	***n*-hexane**	**EtOAc**
TPC (mg GAE[a]/g extract)	141,72±0,56	133,61±0,45	94,51±0,08	107,84±2,04
TFC (mg QE[b]/g extract)	31,61±0,59	21,61±1,65	24,76±0,70	17,48±1,02

TPC and TFC values are mean ± standard deviation of three separate experiments.
[a] Gallic acid equivalents and [b] Quercetin equivalents.
MeOH: Methanolic extract, EtOH: Ethanolic extract and EtOAc: Ethyl acetate extract

The IC_{50} value was defined as the concentration of sample that scavenged 50% of the DPPH. It was calculated from the percentage inhibition of DPPH versus the concentrations of the extracts and standards using the exponential equation. The inhibition capacity (IC_{50}) of the positives controls, Trolox and ascorbic acid, was respectively 27.20 and 43.72 mg mL^{-1}. This values were lowest than IC_{50} of leaves extracts. On other hand, The results showed that inhibition capacity of the n-hexane and methanol extract was significantly difference (p < 0,05) than ethanol and ethyl acetate extracts (Table 2). The significant difference of antioxidant capacity between EtOH and MeOH extracts shows that antioxidant activity is not depending directly to phenol and flavonoids content, but also others molecules participate in this functional biological of extracts. Others studies have demonstrated an antioxidant activity moderated than ours results. In effect, Orak and all found that the methanolic extract of *A. unedo* leaves has an IC_{50}=423 µg mL^{-1} [33].

Table 2. IC_{50} (µg mL^{-1}) values of *A. unedo* extracts and standard

Extracts	**IC_{50} (µg mL^{-1})**
MeOH	95.25
EtOH	280.50
n-hexane	73.73
EtOAc	276.15
Trolox	43.72
Ascorbic acid	27.20

MeOH: Methanolic extract, EtOH: Ethanolic extract and EtOAc: Ethyl acetate extract.
Ascorbic acid and Trolox are the standard antioxidants

The DPPH assay is a very common spectrophotometric method to determine the activity of any antioxidant. The advantage of this method is that the antioxidant activity is measured at ambient temperature, and thus, the risk of the thermal degradation of the molecule tested is eliminated [34]. Free-radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation [35]. Ours

results are in consensus with others studies demonstrating the antioxidant activity of *A. unedo* [33,20,36-39]. Generally, the differences of the results obtained by several searches due essentially to type solvents extracts, chemical contents of extracts and method used for evaluating antioxidant activity.

3.4 Antibacterial Activity

Initial screening of the antibacterial activity of the investigated extracts was studied against five tested microorganisms using the agar well diffusion assay. The results obtained (Table 3) showed that extracts of *A. unedo* exhibited an antibacterial effect against Gram negative and Gram-positive tested bacteria; expect *P. aeruginosa* which we see clearly that the antibacterial activity was moderate.

Significant difference of activities of the investigated extracts against the tested bacterial strains was observed (P = 0.000). The *n*-hexanic extract showed the highest activity against all the tested microorganisms, especially against *S. aureus* and *L. monocytogenes* (zones of inhibition ranged from 34.42±0.26 mm to 40±0.19 mm). While, a moderate activity was observed against *P. aeruginosa and E. coli*, with zones of inhibition ranging from 19±0.97 mm to 23±0 mm. Maximum activity was conferred against *S. aureus* 41±0.67 and 40±0,19 mm for EtOH and *n*-hexane extract respectively, while lower activity observed against *E. coli* 21±1.09 mm for EtOH extract.

Furthermore, the micro-titration assays were conducted to determine the MICs and MBCs of extracts. The MICs and MBCs of the extracts against the tested strains are presented in Tables 4 and 5. The MICs and MBCs values confirmed the results obtained by the agar well diffusion method. Hexanique and MtOH extract had the lowest MICs (0.25-0.5 mg mL^{-1}) and MBCs (0.5-0.5 mg mL^{-1}) against *S. aureus*, while *L. monocytogenes* was inhibited at MICs ranging from 0.25 to 1 mg mL^{-1} and MBCs varied between 1 and 4 mg mL^{-1}. Moreover, the EtOH extract was less active against all the tested microorganisms with MICs and MBCs between 1 and 8 mg mL^{-1}. In generally, Gram positive bacteria are more sensitive than Gram negative bacteria [7]. The minor susceptibility of Gram-negative bacteria may be attributed to an outer membrane surrounding the cell wall which restricts diffusion of hydrophobic compounds through the lipo-polysaccharide. Moreover, the periplasmic space contains enzymes, which are able to break down foreign molecules introduced from outside [40].

Table 3. Inhibition zones of extracts against tested bacteria

Extracts	Inhibition zones diameter [a] (mm) (mean values ± SD)			
	Bacteria [b]			
	E. coli	***S. aureus***	***L. monocytogenes***	***P. aeruginosa***
MtOH	12±0,91	14±0,72	27±1,04	13±0,95
EtOH	9±1,35	17±0,51	41±0,67	-
EtOAc	21±1,09	14±0,19	38±0,84	-
n-hexane	23±0,00	34±0,26	40±0,19	19±0,97

[a] *The diameter of the well (Ø=8 mm) was included*
[b] *Final bacterial density was around 10^6 CFU mL^{-1}*

Table 4. Minimal inhibitory concentration (MIC) of extracts against tested bacteria

Extracts	MIC [a] (mg mL^{-1})			
	Bacteria [b]			
	E. coli	***S. aureus***	***L. monocytogenes***	***P. aeruginosa***
MtOH	8	0,5	0,5	0,5
EtOH	>8	1	1	8
EtOAc	-	-	-	-
n-hexane	2	0,25	0,25	1

[a] *MIC: minimum inhibitory concentration (as mg mL^{-1}).*
[b] *Final bacterial density was around 10^6 CFU mL^{-1}*

Table 5. Minimal bactericidal concentrations (MBC) of extracts (mg mL^{-1}) against tested bacteria

Extracts	MBC [a] (mg mL^{-1})			
	Bacteria [b]			
	E. coli	***S. aureus***	***L. monocytogenes***	***P. aeruginosa***
MtOH	>8	0,5	1	2
EtOH	>	4	4	>8
n-hexane	4	0,5	1	1

[a] MBC: minimum bactericidal concentration (as mg mL^{-1}).
[b] Final bacterial density was around 10^6 CFU mL^{-1}

Also from this study, it appears that Gram-positive bacteria are more sensitive to this oil than Gram-negative bacteria, likewise some strain tested have a big inhibition zone but their MIC is lower or the contrary as reported that the bacteria demonstrating the biggest inhibition zones by diffusion method are not always the one that present the lowest MIC [41]. Antibacterial activity was also reported with leaves extracts of *A. unedo* [42]. In other study conducted by Orak and all, the strawberry tree leaves methanolic extract has exhibited antibacterial activity against *Staphylococcus aureus* but there was no inhibitory effect against *Escherichia coli* [33]. A wide variety of phenols and flavonoids are known to possess antimicrobial properties and in many cases, this activity is due to the presence of active constituents. Bacteria are major source for food industry contamination. The use of natural agents as plants extracts could prevent and/or minimize these contaminations.

4. CONCLUSION

In this study, *A. unedo* leaves collected from North-west of Morocco has showed significant antibacterial and antioxidant activity and could be therefore a useful source in the discovery of new antibacterial and antioxidant compounds. On other hand, this application in food functional industry is strongly recommended as new agent for conservation. Others studies on antibacterial and antioxidant activities of *A. unedo* leaves extract in food system should be made in future.

ACKNOWLEDGEMENTS

This study was supported by the Centre National pour la Recherche Scientifique et Technique (CNRST) from Morocco. We wish to express our gratitude to prof. Abdeslam ENNABILI, PAMSN laboratory, National Institute of Medicinal and Aromatic Plants, Sidi Mohamed ben Abdellah University, Fes, Morocco for further identification of plant. We thank all people having shared with us their knowledge, for their encouragement and support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pietta PG. Flavonoids as antioxidants. Journal Natural Product. 2000;63:1035-1042.
2. Halliwell B. Antioxidants in human health and disease. Annual Reviews. 1996;16:33-50.
3. La Vecchia C, Altieri A, Tavani A. Vegetables, fruit, antioxidants and cancer: A review of Italian studies. European Journal of Nutrition. 2001;40:261-267.
4. Nathan C. Antibiotics at the crossroads. Nature. 2004;21:899-902.
5. Akins RL, Haase KK. Gram-positive resistance: Pathogens, implications and treatment options: Insights from the Society of infectious diseases pharmacists. Pharmacotherapy. 2005;25:1001-1010.
6. Cornaglia G. Fighting infections due to multidrug-resistant Gram-positive pathogens. Clin Microbiol Infect. 2009;15:209-211.
7. Bouyahya A, Abrini J, El-Baabou A, Bakri Y, Dakka N. Determination of phenol content and antibacterial activity of five medicinal plants ethanolic extracts from North-West of Morocco. J Plant Pathol Microbiol. 2016;7:107-111.
8. Bouyahya A. Alicaments: des aliments aux médicaments, quel apport pour la santé? Annales des Sciences de la Santé. 2016;1:1-3.
9. Lin CC, Huang PC. Antioxidant and hepatoprotective effects of *Acanthopanax*

senticosus. Phytother. Res. 2002;14:489-494.

10. Perry EK, Pickering AT, Wang WW, Houghton PJ, Perru NS. Medicinal plants and Alzheimer's disease: From ethnobotany to phytotherapy. J. Pharm. Pharmacol. 1999;51:527-534.
11. Schinella GR, Tournier HA, Prieto JM, Mordujovich de Buschiazzo P, Ríos JL. Antioxidant activity of anti-inflammatory plant extracts. Life Sci. 2002;70:1023-1033.
12. Velázquez E, Tournier HA, Mordujovich de Buschiazzo P, Saavedra G, Schinella GR. Antioxidant activity of Paraguayan plant extracts. Fitoterapia. 2003;74:91-97.
13. Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, Benjelloun W. Phytotherapy of hypertension and diabetes in oriental Morocco. J. Ethnopharmacol. 1997;58:45–54.
14. Tahraoui A, El-Hilali J, Israili ZH, Lyoussi B. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in the south-eastern Morocco (Errachidia province). J. Ethnopharmacol. 2007;110:105–117.
15. Ziyyat A, Boussairi E. Cardiovascular effects of *Arbutus unedo* L; in spontaneously hypertensive rats. Phytother. Res. 1998;12:110–113.
16. Ziyyat H, Mekhfi M, Bnouham A, Tahri A, Legssyer J, Hoerter R. Fischmeister *Arbutus unedo* induces endothelium dependent relaxation of the isolated rat aorta. Phytother. Res. 2002;16:572.
17. Rosato JA, Porras T, Conde M, Sage SO. Cyclic nucleotides modulate store-mediated calcium entry through the activation of protein-tyrosine phosphatases and altered actin polymerization in human platelets. J. Biol. Chem. 2001;276:15666–15675.
18. Redondo PC, Ben-Amor N, Salido GM, Bargeti A, Pariente JA, Rosado JA. Ca^{2+} independent activation of Burton's tyrosine kinase is required for store-mediated Ca2+ entry in human platelets Cell. Signal. 2005;17:1011–1121.
19. Redondo PC, Jardin I, Hernandez-Cruz JM, Pariente JA, Salido GM, Rosado JA. Hydrogen peroxide and peroxynitrite enhance Ca2+ mobilization and aggregation in platelet from type 2 diabetic patients. Biochem. Biophys. Res. Commun. 2005;334:779–789.
20. Oliveira I, Coelho V, Baltasar R, Pereira JA, Baptista P. Scavenging capacity of strawberry tree (*Arbutus unedo* L.) leaves on free radicals. Food Chem. Toxicol. 2009;47:1507–1511.
21. Singleton V, Orthofer R, Lamuela-Raventos RA. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology. 1999; 299:152-175.
22. Brighente IMC, Dias M, Verdi LG, Pizzolatti MG. Antioxidant activity and total phenolic content of some Brazilian species. Pharmaceutical Biology. 2007;45:156-61.
23. Kubola J, Siriamornpun S. Phenolic content and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf stem and fruit fraction extracts *in vitro*. Food Chem. 2008;110:881-890.
24. Ismaili H, Milella L, Fkih-Tetouani S, Ilidrissi A, Camporese A, Sosa S, Altinier G, Loggia RD. *In vivo* topical anti-inflammatory and *in vitro* antioxidant activities of two extracts of *Thymus satureioides* leaves. J Ethnopharmacol. 2004;91:31–36.
25. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocher P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry. 2006;97:654–660.
26. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry. 2001;49:5165–5170.
27. Laranjinha J, Vieira O, Madeira V, Almeida L. Two related phenolic antioxidants with opposite effects on vitamin E content in low density lipoproteins oxidized by ferrylmyoglobin: Consumption versus regeneration. Archives of Biochemistry and Biophysics. 1995;323:373–381.
28. Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol. J. Environ. Stud. 2006;15:523–530.
29. Das NP, Pereira TA. Effects of flavonoids on termal autoxidation of palm oil: Structure–activity relationship. Journal of the American Oil Chemists' Society. 1990;67:225–258.
30. Pavlović DR, Lakušić B, Došlov-Kokoruš Z, Kovaćević N. Arbutin content and

antioxidant activity of some *Ericaceae* species. Pharmazie. 2009;64:656–659.
31. Male Ž, Plazibat M, Vundać VB, Žuntar I. Qualitative and quantitative analysis of flavonoids of the strawberry tree—*Arbutus unedo* L. (Ericaceae). Acta Pharm. 2006; 56:245–250.
32. Mendes L, de Freitas V, Baptista P, Carvalho P. Comparative antihemolytic and radical scavenging activities of strawberry tree (*Arbutus unedo* L.) leaf and fruit. Food Chem. Toxicol. 2011;49:2285–2291.
33. Orak HH, Yagar H, Isbilir SS, Demirci AŞ, Gümüş T, Ekinci N. Evaluation of antioxidant and antimicrobial potential of strawberry tree (*Arbutus unedo* L.) leaf. Food Sci. Biotechnol. 2011;20:1249–1256.
34. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. LWT-Food Sci. Technol. 1997;30: 609–615.
35. Dvorakova M, Moreira MM, Dostalek P, Skulilova Z, Guido LF, Barros AA. Characterization of monomeric and oligomeric flavan-3-ols from barley and malt by liquid chromatography–ultraviolet detection–electrospray ionization mass spectrometry. J. Chromatogr. A. 2008; 1189:398–405.
36. Pabuçcuoğlu A, Kıvcak B, Bas M, Mert T. Antioxidant activity of *Arbutus unedo* leaves. Fitoterapia. 2003;74:597–599.
37. Pavlović DR, Branković S, Kovačević N, Kitić D, Veljković S. Comparative study of spasmolytic properties, antioxidant activity and phenolic content of *Arbutus unedo* from Montenegro and Greece. Phytother. Res. 2011;25:749–754.
38. Boulanouar B, Abdelaziz G, Aazza S, Gago C, Miguel MG. Antioxidant activities of eight Algerian plant extracts and two essential oils. Ind. Crop Prod. 2013;46:85–96.
39. Malheiro R, Sá O, Pereir E, Aguiar C, Baptista P, Pereira JA. *Arbutus unedo* L. leaves as source of phytochemicals with bioactive properties. Ind. Crops Prod. 2012;37:473–478.
40. Laciar A, Ruiz ML, Flores RC, Saad JR. Antibacterial and antioxidant activities of the essential oil of *Artemisia echegarayi* Hieron. (Asteraceae). Rev Argent Microbiol. 2009;41:226-231.
41. Akrout A, El Jani H, Amouri S, Neffati M. Screening of antiradical and antibacterial activeities of essential oils of *Artemisia campestris* L., *Artemisia herba* alba Asso, and *Tymus capitatus* Hoff. Et Link. Growing wild in the southern of Tunisia. Res Sci Technol. 2010;2:29-39.
42. El Ouarti A, Haouat AC, Sqalli H, Haggoud A, Ennabili A, Ibnsouda S, Iachagar M, Iraqui M. Extra- and intracellular antimycobacterial activity of *Arbutus unedo* L. Afr. J. Microbiol. Res. 2012;6:1283–1290.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15247